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14. ABSTRACT

The goal of our program was to access the viability of using MEMS devices to detect firing signatures from neurons. In order to evaluate these devices we needed to prepare and test MEMS devices biologically relevant situations and develop systems that will be sensitive to the rising and falling levels of potassium, present during neural activity. Several different MEMS structures were tested and one was found that gave a measurable resonance signal in water with ionic strength comparable to biological systems. The MEMS device showed efficient cation binding, however it was not selective to potassium. A potassium specific crown ether surface treatment was applied to the MEMS device, however this surface treatment did not lead to selective potassium binding. We are currently examining the surface coating to determine if the crown ether density was too low for efficient potassium binding. In a parallel effort we have developed methods for selective cell binding to TiN surface. Cell adhesion molecules were anchored to the TiN surface, promoting the adhesion of dissociated cells.

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Summary

The goal of this investigation was to test the viability of film bulk acoustic resonator MEMS devices as sensors of neuronal activity. There were several key questions that needed to be answered in this regard. We laid out three tasks to be performed in this program:

1. Investigate the interaction of MEMS devices (film bulk acoustic resonator structures) with neuronal tissue. Will the neuronal tissue affect the resonant absorption frequency of an untreated device?
2. Derivatize the surfaces of MEMS devices to make them sensitive to neuronal activity indicators (such as potassium ion) and investigate their interaction with active neuronal tissue. Can devices be prepared with high enough sensitivity to make them useful for use in a NAS?
3. Derivatize the surfaces of emal electrodes and MEMS devices with neuron specific binding groups and investigate the interaction of these derivatized MEMS devices with active neuronal tissue. Can increasing the degree of interaction between the MEMS device and the neuron increase the sensitivity to neuronal activity?

In order to carryout these tasks we have built fbar-MEMS devices and tested them. The devices to be tested here were of a new design that allowed for direct access of the device to a tissue sample. This required a complete rework of the design of the MEMS devices and took a significant amount of time and effort to generate the devices. The devices were built on silicon substrates. The substrate had > 50 individual devises, with various sizes and shapes. This range of devices was needed to determine the optimal shape and size of device for tissue testing.

The initial designs of these devices had an exposed electrode, which could be put in direct contact with the tissue sample. The devices have good signal to noise and for rf absorption in air, but gave very poor signals when in contact with water or a tissue sample. The fluid medium damped out the MEMS acoustic oscillations killing the signal. Over-coating the device with a thin film of parylene led to devices which gave an excellent signal, when in contact with the tissue sample. This study completed step 1. of our desired goals.

The water stable and active devices were treated with solutions of sodium, potassium and cesium salts. The carbonate and bicarbonate salts gave a strong MEMS response, with the shift in resonance frequency being roughly proportional to the mass of the cations (i.e. Na > K > Cs). This response was highly pH dependent, however, only giving a measurable response at pH levels above 8, with an optimal pH > 10. Thus the MEMS device is a very useful mass sensor, but it must be treated to achieve efficient discrimination of potassium from the background of other ions. Moreover, the active pH range must be shifted to the physiologically relevant range (*i.e.* 7.0 - 7.4).

We have prepared crown ether complexes and bound them to the active MEMS surface. The crown ethers selectively bind potassium ion over sodium, with a high level of specificity. Unfortunately, these crown treated devices give low MEMS signals and poor discrimination between sodium and potassium. The problem here is a low surface

concentration of the crown ether. We are working now to increase the surface density of crown ethers.

The third goal in our program was the selective binding of neurons to metal electrode materials. The MEMS device has a top metal electrode that will be used to anchor the MEMS device to the tissue sample. In order to accomplish this we need to develop the technology for selectively binding neurons to metal surfaces. This program has only been done at metal and metal-nitride surface and not applied to MEMS devices at this time. This has been accomplished by first treating the metal surface with alkylthio-carboxylic acids ($HS-(CH_2)_n-COOH$), followed by exposure to antibodies (specific to neuron proteins) and amide coupling reagents. The antibodies bind to the surface covalently at high density. We have tested the antibodies with their specific antigen and found that > 50% of the surface bound antibodies are active. In a parallel set of experiments we have demonstrated that cell adhesion molecules can also enhance the binding of neurons to inorganic surfaces.

The details of all of these experiments are given in the pages below.

Program Goals

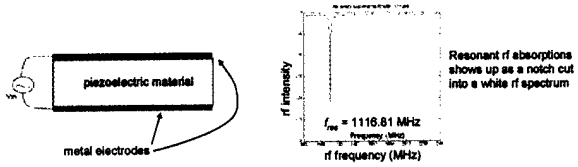
Thompson, Berger, Baudry, Kim, McKenna

- Neural activity sensor (NAS)
  - Sensor must reliably monitor activity at one or a small cluster of neurons
    - many sensors active without overlapping signals.
    - communicate with the sensor via a wireless link
    - stable *in vivo*
  - fbar-MEMS as sensor elements
- Selective binding of neurons to electrode/sensor surfaces
  - Surface coatings for neuron adhesion
    - enhanced stability and communication between neuron and electrode/sensor
  - Adhesion coatings for other cells in neural tissue
    - binding will give greater positional stability for implanted devices
  - Systems:
    - NAS will need to be bound to the neuron it is sensing
    - multielectrode arrays will be treated to enhance communication and stability

Sensing Element of NAS

Thompson, Berger, Baudry, Kim, McKenna

- Film bulk acoustic wave resonator MicroElectroMechanical Systems (fbar-MEMS)
  - devices absorb rf radiation at a fixed resonant frequency, f_{res}
  - currently used as rf notch filters in cellular phones
- The resonant frequency of MEMS can be readily tuned.
  - the resonant frequency is controlled by setting the thickness of the ZnO film, $f_{res} \propto d$.
  - for the ZnO thickness range given, the resonant frequency will range from 1 to 10 GHz.
- Resonant frequency is also affected by the environment.



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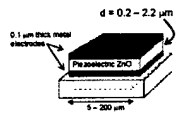
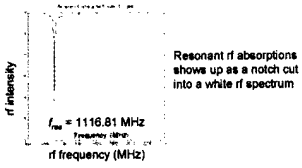


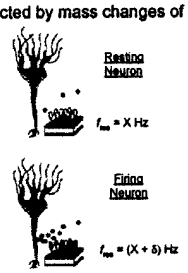
Acoustic wave MEMS with air pocket for added sensitivity



Amplifying MEMS Sensitivity to Neuron Activity

Thompson, Berger, Baudry, Kim, McKenna

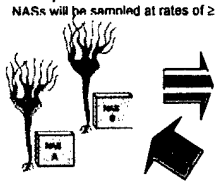
- MEMS are sensitive to environmental changes
  - MEMS device will be affected by the chemical/electrical cellular membrane ionic changes surrounding the neuron at firing.
- MEMS resonant frequencies are markedly affected by mass changes of the electrodes.
  - $\Delta f / f_{res} \approx -C_m \Delta m$
    - $\Delta m \propto$ change in ion binding at electrodes
    - Δf = frequency shift
    - C_m = proportionality constant (depends on device structure)
  - Crown ethers will selectively (*e.g.* K^+ *vs* Na^+) trap ions released during neuron firing. The kinetics of potassium uptake and release are rapid.

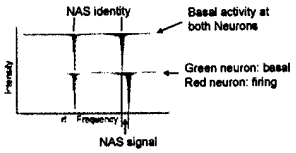


Multisite Monitoring Neuronal Activity Sensors (NASs)

Thompson, Berger, Baudry, Kim, McKenna

- The NAS with a wide range of different basal frequencies (f_{res}) allow probing of many neurons simultaneously.
  - The change in f_{res} > firing induced shift (NAS signal), different signals will be readily separated.
- NASs will be sampled at rates of ≥ 1 KHz.



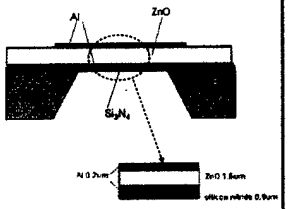


Site specific binding of NASs will allow the simultaneous monitoring of different types of neurons.

Diaphragm-Supported fbar

Thompson, Berger, Baudry, Kim, McKenna

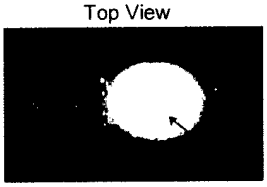
- Typical fbar is
  - ➢built on silicon nitride diaphragm
  - ➢with a piezoelectric ZnO film and two Al layers.
- fbar with 1.6 μm thick ZnO
  - ➢resonates at 1 GHz.



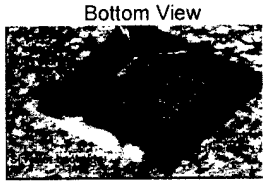
Fabricated fbar-MEMS

Thompson, Berger, Baudry, Kim, McKenna

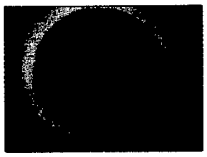
Top View

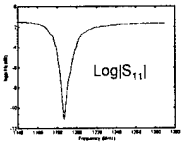


Bottom View



fbars on 3' Silicon Wafer →





Testing Set-up for Sensing in Liquid

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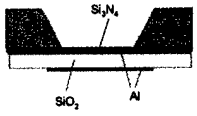
- ❑ fbar is mounted upside down.
- ❑ Liquid is placed in the cavity in the fbar wafer.



fbar Quality Factors in Air and Liquid

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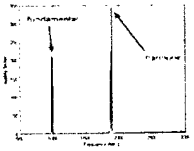
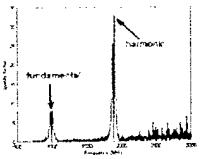
Q in Air



Q in Water



- Quality factor Q = stored energy / dissipated energy per cycle
  Larger Q, better fbar performance
- Q Calculation from measurement of S_{11}
  Q = resonant frequency / -3dB bandwidth of S_{11}

Liquid Mass Sensing with fbar

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- Q drops in water, due to large acoustic energy loss to the water.
- SiN-diaphragm supported fbar
  - with a Q of 33, detecting a 5~10 ppm shift resonant frequency in water is possible.
    ⇒ thus the minimum detectable mass change is 10^{-8}g/cm^2
    (70 Å^2/bound-K$^+$ or 3-5% of a K$^+$ monolayer)
  - unfortunately, there is no shift in v_f with changing ion concentration for SiN supportd fbar-MEMS
- fbar coated with TiO_2
  - gives the potential for direct ion binding *via* surface OH/O$^-$ groups
  - allows for surface coating (crown ether)

Resonant Frequency Shift with Positive Ions

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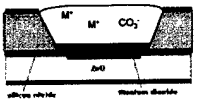
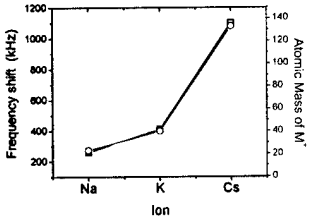
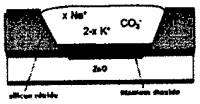


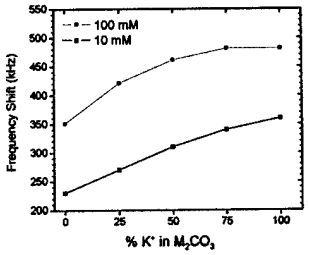


- Solutions consist of 100 mM M_2CO_3 (M = Na, K, Cs)
- Frequency shift relative to pure water
- Heavier positive ion gives larger resonant frequency shift

Frequency Shift in Na^+ and K^+ Mixture

Thompson, Berger, Baudry, Kim, McKenna

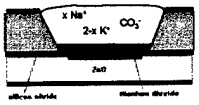


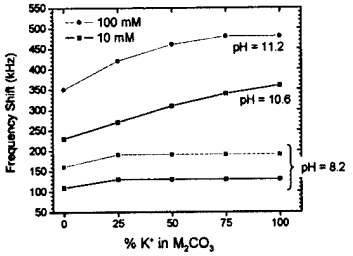


- MEMS device treated with Na_2CO_3/K_2CO_3 mixtures
- Total [M_2CO_3] kept constant at 10 or 100 mM
- 100 mM solution saturates at > 75% K$^+$
- 10 mM: pH = 10.6; 100 mM: pH = 11.2

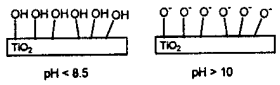
Frequency Shift in Na^+ and K^+ Mixture

Thompson, Berger, Baudry, Kim, McKenna



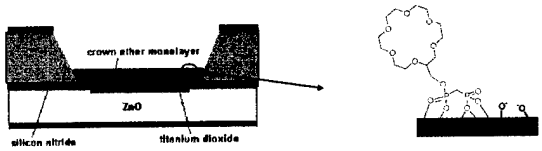


- pH was lowered by adding HCl
- Sensitivity is very low in at pH = 8
- Signal saturates at 25% K^+



Crown Ether on fbar

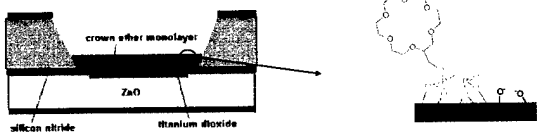
Thompson, Berger, Baudry, Kim, McKenna



- Monolayer crown ether is bound to TiO_2 surface through phosphonate ligand.
- This crown ether ring is designed to efficiently bind only potassium ions

Crown Ether on fbar

Thompson, Berger, Baudry, Kim, McKenna



- Similar results with and without crown ether coating
  - Frequency shifts for Na_2CO_3/K_2CO_3 mixtures reflect only the difference in mass of the ions, no selective K binding observed
  - Response time of the sensor with crown ether is 5-10 times faster than for bare TiO_2

Crown Ether on fbar

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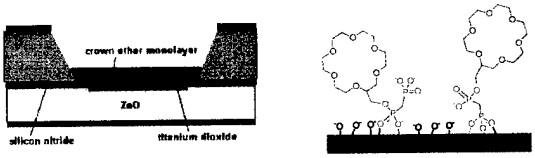

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Part 2: Binding MEMS/electrode to the Neuron

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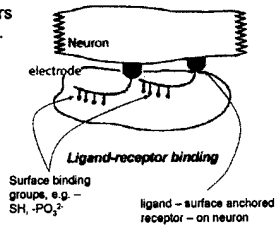
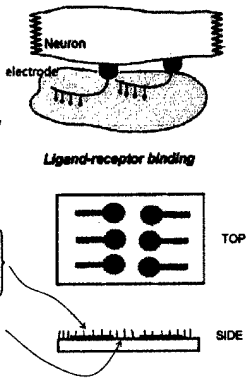
- MEMS placed near neurons may not stay there
- The NAS will bind to neurons specifically and spontaneously.
  - Specific groups (ligands, antibodies, *etc.*) will be designed to bind to the surfaces of neurons, at predetermined sites.
  - Binding can be specific for receptors on the surfaces of chosen neurons.
  - Tether will anchor MEMS to the neuron.
- Strong bonding will lead to high stability in chronic applications.
- Potential ligand-receptor: antibodies and cell adhesion molecules



Strong neuron-surface bonding in multielectrode arrays

Thompson, Berger, Baudry, Kim, McKenna

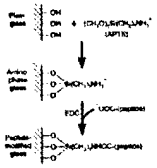
- Multisite electrode arrays
  - electrode arrays are used extensively for simultaneous monitoring of many neurons at once
  - current techniques involve physically placing the tissue sample on the array
    - problems:
      - may give poor electrical contact
      - poor stability, tissue sample is free to move over time
  - Our goals:
    - Develop selective chemistries to form strong contacts between neuron and electrical pads of array (red lines)
    - Develop chemistries to bind tissue unselectively to glass or other substrates (blue lines), to act as an anchor to hold the tissue sample in place

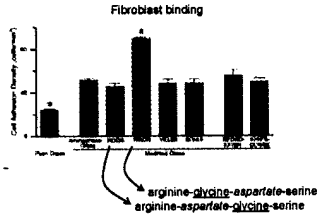


The use of cell adhesion molecules (CAM) to bind neurons

Thompson, Berger, Baudry, Kim, McKenna

- Different peptides can be used to target binding of different cells
  - specific sequences have been used for selective binding of neurons, astrocytes, fibroblasts, osteoblasts, *etc.*, to rigid substrates (typically glass)
  - CAM recognized by integrin proteins
  - can be used to target different parts of a given cell type by targeting the specific membrane bound proteins





R. Bizios, *et. al.*, *Biomat.*, **2002**, *23*, 511-5.

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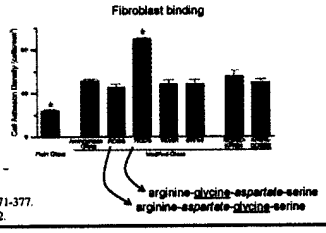
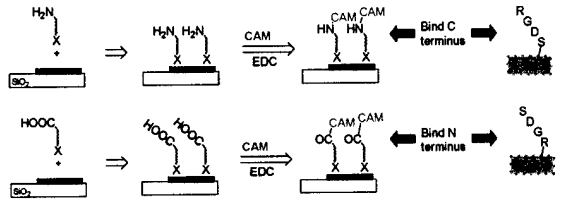
Cell type	peptide
neuron	IKVAV, YIGSR
fibroblast	RGDS
astrocytes	KHIFSDDSSE
osteoblasts	KRSR



R. Bizios, *et. al.*, *Biomat.*, **2002**, *23*, 511-5.
R. Bizios, *et. al.*, *J. Biomed. Mater. Res.*, **1998**, *40*, 371-377.
K.E. Healy, *et. al.*, *Biotechnol. Prog.*, **1999**, *15*, 19-32.

Electrode surface coating

Thompson, Berger, Baudry, Kim, McKenna



- Substrate/electrode anchoring groups will spontaneously react/anchor to specific surfaces
  - e.g. Cl_3Si- on silicon or glass, HS- on gold, H_2O_3P- on TiN/Ti
- Changing chemistry of the surface groups allows us to probe CAM orientational affects: RGD is what is recognized by integrin

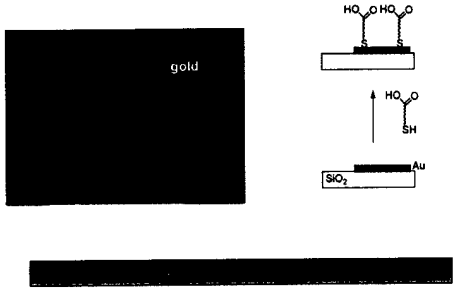
Protocol for Culturing Dissociated Hippocampal Cells

Thompson, Berger, Baudry, Kim, McKenna

- The hippocampi of E-18 rats are dissected and mechanically dissociated after treatment with 1% trypsin.
- Cells are cultured in Neuro Basal Medium (Gibco) with 0.5 mM glutamine, B-27 Supplement, pen-strep, and for the first few days, 25 mM glutamate.
- The cells are cultured in the presence of the substrate at about 125,000 cells/cm², in a 10% CO_2 incubator and fed twice a week.

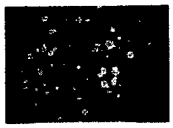
Cell attachment on untreated glass, SAM-gold

Thompson, Berger, Baudry, Kim, McKenna



Cell attachment/growth with only SAM/EDC

Thompson, Berger, Baudry, Kim, McKenna



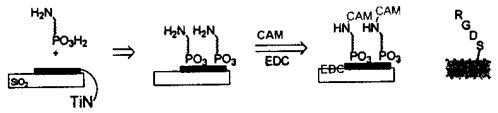
Cells 11 days old on SAM-EDC
Before fixing.

After fixing, viewed from top of the sample (gold side up)
1. dead cells.
2. strong attachment on gold and glass.
3. bald spot-no cell attachment.

- EDC treatment leads to efficient nonspecific cell attachment
- Similar levels of binding and neuron growth on glass and SAM treated Au.

Peptide anchoring to electrode surface

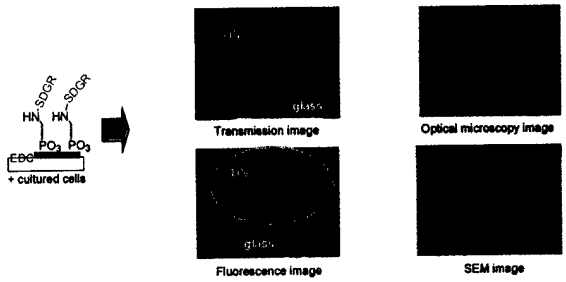
Thompson, Berger, Baudry, Kim, McKenna



- Example shown for TiN electrode, readily adapted to TiO_2
  - phosphonate groups bind strongly to Ti and TiO_2 surfaces
  - similar surface treatment has been used in long term bone implants, minimal loss of the phosphonate surface coating
  - Simultaneously developing coatings for MEMS and electrode arrays (TiO_2 *vs.* TiN)
- Efficient binding of $R\text{-}PO_3^{2-}$ groups to TiN and Ti substrates is observed
  - surface coverage estimated by contact angle and XPS measurements
  - tolerant to a wide range of function groups on R group, *i.e.* $-NH_2$, -COOH, peptide
  - method will prove very useful for selective derivatization of Ti or TiN electrodes without contamination of the substrate

Cell binding adhesion to CAM treated TiN

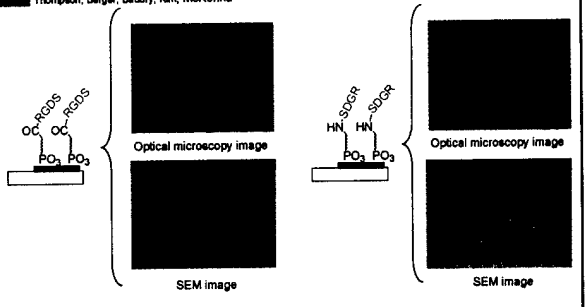
Thompson, Berger, Baudry, Kim, McKenna



- Untreated TiN and glass give only clusters of dead cells
- Neuron binding and growth on CAM treated surface

Cell binding adhesion to CAM treated TiN

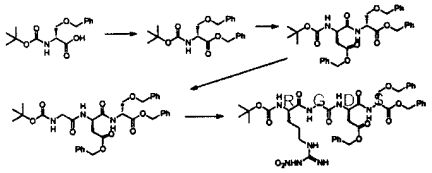
Thompson, Berger, Baudry, Kim, McKenna



- Carboxylic acid treated surface does not lead to cell binding
  - RGDS bound backward
  - Free surface groups (-COOH) do not promote cell adhesion

Synthesis of CAM peptide (RGDS)

Thompson, Berger, Baudry, Kim, McKenna



- Synthesized tetrapeptide (RGDS)
  - all side groups as well as C and N termini protected with orthogonal groups
  - we can selectively anchor either terminus with no side group attachment
  - Long tether can be added to the C or N terminus
- CAM surface binding
  - can be bound to amine or carboxyl groups already at the surface (described above)
  - can be bound to phosphonate groups first and then anchored to TiN or Ti surface

Polypeptide primary structures

Thompson, Berger, Baudry, Kim, McKenna

Ile-Lys-Val-Ala-Val
(isoluecine-lysine-valine-alanine-valine)

Tyr-Ile-Gly-Ser-Arg
tyrosine-isoluecine-glycine-serine-arginine

Arg-Gly-Asp-Ser
arginine-glycine-aspartate-serine

Lys-His-Ile-Phe-Ser-Asp-Asp-Ser-Ser-Glu
lysine-histidine-isoluecine-phenylalinine-serine-
-aspartate-aspartate-serine-serine-glutamate

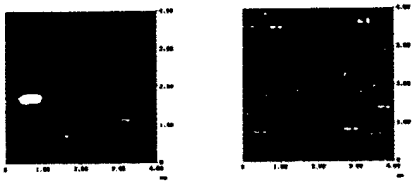
Lys-Arg-Ser-Arg
lysine-arginine-serine-arginine

Cell type	peptide
neuron	IKVAV, YIGSR
fibroblast	RGDS
astrocytes	KHIFSDDSSE
osteoblasts	KRSR

R. Bizios, *et. al.*, *Biomat.*, 2002, *23*, 511-5.
R. Bizios, *et. al.*, *J. Biomed. Mater. Res.*, 1998, *40*, 371-377.
K.E. Healy, *et. al.*, *Biotechnol. Prog.*, 1999, *15*, 19-32.

AFM studies of bound anitbodies

Thompson, Berger, Baudry, Kim, McKenna

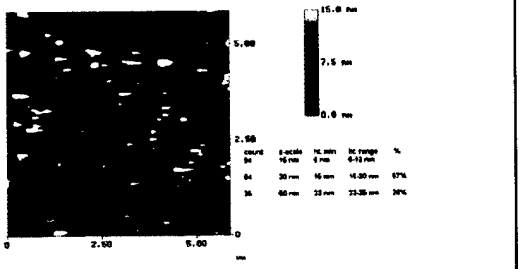


unactivated gold + antibodies

activated gold + antibodies

- Atomic force microscope images show bound antibody
  - the white dots are high spots on the gold substrate
  - the height (7 nm) is consistent with the size expected for the antibody
- Once they are bound are they active?
  - bound antibodies were treated with the antigen

Thompson, Berger, Baudry, Kim, McKenna



- *ca.* 50% of the anitbodies show an increase in size, consistent with antigen binding
  - no change if the wrong antigen is used
  - Antibodies are still active!
- Colorometric assay was tried, but the concentration was too low for detection.